Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BIP01/99/PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP00/10030	International filing date (*day/month/year*) 11 October 2000 (11.10.00)	Priority date (*day/month/year*) 11 October 1999 (11.10.99)
International Patent Classification (IPC) or national classification and IPC G01N 21/00		
Applicant BITTNER, Christoph		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 10 May 2001 (10.05.01)	Date of completion of this report 02 May 2002 (02.05.2002)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP00/10030

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:

pages 1-10, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

☒ the claims:

pages 1-21, as originally filed

pages _____, as amended (together with any statement under Article 19

pages _____, filed with the demand

pages _____, filed with the letter of _____

☒ the drawings:

pages 1/6-6/6, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

☐ the sequence listing part of the description:

pages _____, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-21	YES
	Claims		NO
Inventive step (IS)	Claims	1-21	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-21	YES
	Claims		NO

2. Citations and explanations

1. The present application relates to a method (independent Claim 1) and a device (independent Claim 10) for characterizing a culture liquid by means of microscopic imaging in dark-field illumination. The cells are classified as living or dead on the basis of a comparison between the light intensities at the cell edges and in the cell inner area.

The dependent claims relate essentially to further details of the optical illumination/imaging such as bright illumination, fluorescence, interference, phase contrast, polarization, wavelength selection, pulsed light or the spatial arrangement of the illumination arrangement, the microscope objective and the image-evaluating device relative to the culture liquid.

Observations - Claim 10:

i) The term "dark-field illumination" refers to a particular arrangement of the light source, specimen and detector, and therefore a device that does not include the shape and position of the specimen cannot be further defined with this term.

ii) Even if the culture liquid, including the cells, is not a feature of the device, the way in which the image-evaluating device works is defined sufficiently clearly to a person skilled in the art; said device must be configured in such a way that it identifies the "cells" in the image and classifies them into two groups by comparing their intensities at the edge and in the inner area.

2. This report makes reference to the following documents:

D1: DE 40 32 002
D2: EP 0 277 789
D3: WO 87/04247.

D1 (Figures 1 and 4) is cited in the description of the present application and discloses a microscope probe and a measuring method in which cells of a culture broth are monitored optically in a bioreactor and counted by means of microscopic imaging on a video camera. The concentration of living cells is determined by means of calibration of the specimen volume by an image-processing device. The living cells are differentiated from the measurement background (residues, contamination and air bubbles) by exceeding a particular threshold value for edge characteristic, sharpness or contrast within the digital image processing. Furthermore, living cells have "a content of particularly strongly fluorescent coenzymes" which clearly distinguishes these cells from the remaining medium by fluorescence. Figure 4 shows an "incident illumination arrangement" having light source 50, specimen 4 and detector 51 and

Figure 1 shows a transmitted illumination arrangement with a light source (not shown, but coupled to the inlet of the optical waveguide 30), specimen 4 and a detector (not shown, but arranged to the right of window 2). The arrangements according to Figure 4 do not, however, correspond to a dark-field illumination, since it separates the incoming beams and the outgoing beams by means of a dichroitic filter 53 instead of the geometrical arrangement of the reflected beam cluster and the detector.

3. NOVELTY

D1 is the closest prior art with regard to independent Claims 1 and 10.

D1 discloses all the features of Claims 1 and 10 except for:

a) the dark-field illumination;

b) an image-evaluating device that carries out a comparison between the light intensities at the cell edges and the cell inner areas (in order to classify the cells as living or dead).

Independent Claims 1 and 10 and dependent Claims 2 to 9 and 11 to 21 therefore meet the requirement of novelty (PCT Article 33(2)).

4. INVENTIVE STEP

Regarding a):
Next to the incident and transmitted illumination arrangement, dark-field illumination and bright-field

illumination are the illumination alternatives best known to a person skilled in the art. On the basis of the arrangement of the optical components in D1, these illumination alternatives could be easily applied.

Regarding b):

D1 does not suggest that the image-processing device is configured or is intended to be configured for comparing light intensities at different points of a "cell".

D2 (Figure 1, page 1) relates predominantly to an arrangement for capacitive determination of a biomass and mentions only optical scattered light measurements which are generally known in the field, without providing details about the shape of the pictured cells.

D3 (Figures 4 and 5) discloses an arrangement for determining the fluorescence of a biomass by means of evanescent excitation; there is no cell imaging.

In light of feature b), independent Claims 1 and 10 and dependent Claims 2 to 9 and 11 to 21 therefore meet the requirement of inventive step (PCT Article 33(3)).